



Research article

Seasonal changes in morphophysiological traits of two native Patagonian shrubs from Argentina with different drought resistance strategies

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ARTICLE INFO

Keywords:

Avoidance
Dry periods
Ecophysiology
LC/MS-MS
ABA
ABA-GE
IAA
GAs
Semi-arid region
Tolerance

ABSTRACT

In semi-arid regions, plants develop various biochemical and physiological strategies to adapt to dry periods. Understanding the resistance mechanisms to dry periods under field conditions is an important topic in ecology. *Larrea divaricata* and *Lycium chilense* provide various ecological services. The aim of this work is to elucidate new morpho-histological, biochemical and hormonal traits that contribute to the drought resistance strategies of two native shrubs.

Green leaves and fine roots from *L. divaricata* and *L. chilense* were collected in each season for one year, and various traits were measured. The hormone (abscisic acid, ABA-glucose ester, gibberellins A₁ and A₃, and indole acetic acid) contents were determined by liquid chromatography coupled to mass spectrometry. Rainfall data and the soil water content were also measured. A multivariate analysis showed that green leaves from *L. divaricata* showed high values for the leaf dry weight, blade leaf thickness and ABA content in the summer compared with those from *L. chilense*. Fine roots from *L. divaricata* had high RWC and high IAA levels during the autumn-dry period compared with those from *L. chilense*, but both had similar levels during the winter and spring.

Our results support the notion that species with different drought resistance mechanisms (avoidance or tolerance) display different responses to dry periods throughout the year. *Larrea divaricata*, which exhibits more xerophytic traits, modified its morphology and maintained its physiological parameters (high RWC in leaves and roots, high ABA levels in leaves during summer, high GA₃ in leaves and high IAA in roots during autumn) to tolerate dry periods, whereas *Lycium chilense*, which displays more mesophytic traits, uses strategies to avoid dry periods (loss of leaves during autumn and winter, high RWC in leaves, high ABA-GE and GA₃ in leaves during summer, high GA₁ and GA₃ in roots during summer, and high IAA in roots during autumn and summer) and thus has a metabolism that is more dependent on water availability for growth.

1. Introduction

In semi-arid ecosystems, water availability is one of the main factors controlling plant populations and biological activity (Schwinning and Sala, 2004), and access to water affects the physiological state and metabolic processes of vegetation (Yan et al., 2000). Thus, the amount of water added to soil through rainfall and its effects on physiological traits might be important factors in determining plant responses to drought (Devitt et al., 1997). Species from semi-arid regions have developed different resistance strategies to cope with water stress,

classified into two main groups: drought-avoiding plants and drought-tolerant plants (Levitt, 1980). In general drought-avoiding species are characterized by deciduous phenology, high/faster metabolism, high potential for resource capture, and low investment in secondary metabolites (Dolferus, 2014; Cenzano et al., 2016). Drought-tolerant plants, can maintain their normal parameters during long periods of drought using strategies such as osmotic adjustment, high elastic modulus, maintenance of root growth and water uptake, antioxidant mechanism and production of secondary metabolites with various functions (Seo and Park, 2011; Cenzano et al., 2016; Varela et al.,

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<https://doi.org/10.1016/j.plaphy.2018.03.018>

Received 6 December 2017; Received in revised form 11 March 2018; Accepted 16 March 2018

Available online 19 March 2018

0981-9428/ © 2018 Published by Elsevier Masson SAS.

2016).

It has been shown that species with different morphological and functional traits have a variety of adaptive strategies related to water and nutrient conservation (Bertiller et al., 2005). Also, biochemical studies had shown variable responses in the pigment content under drought conditions (Reynolds et al., 2005; Cenzano et al., 2013) and hormones regulation to adapt to environmental changes (Seo and Park, 2009; Peleg and Blumwald, 2011). Abscisic acid (ABA) regulates a plant's water balance, growth and development, as well as stress tolerance (Tuteja and Sopory, 2008; Finkelstein, 2013) serving as a bridge between avoidance and tolerance responses (Jarzyniak and Jasiński, 2014). Also, have been shown that long-distance ABA signalling is mainly controlled by conjugation of the hormone with glucose giving as a product ABA-glucose ester (ABA-GE). ABA-GE is transported from roots to leaves giving free active ABA after their hydrolyzation (Sauter and Hartung, 2002). It is also known that interactions between phytohormones are essential for the proper regulation of growth and developmental processes (Yaish et al., 2010; Xu et al., 2013). For example, indole acetic acid (IAA) is involved in plant morphogenesis, root system development (Zhao, 2010), and modulation of the root architecture (Shi et al., 2014), and IAA increase under drought stress favours drought tolerance (Dobra et al., 2010). Gibberellins (GAs) promote seed germination and vegetative growth (Sun, 2010), and drought stress can also influence the levels of bioactive GAs (Zawaski and Busov, 2014).

The semi-arid Patagonian rangelands in Argentina are dominated by a variety of grass and shrub species with different adaptive strategies to resist drought (Moreno et al., 2010). Two dominant shrubs from the Patagonian Monte are *Larrea divaricata* and *Lycium chilense*. *L. divaricata*, which is deep-rooted and has high potential for resource conservation, is an evergreen species with high polyphenol production and high antioxidant capacity, classified as a drought-tolerant species. *L. chilense*, a shallow-rooted plant with high potential for resource acquisition, is a deciduous species with low polyphenol production and is considered a drought-avoiding species (Bertiller et al., 1991; Campanella and Bertiller, 2008; Varela et al., 2016). The study of these two species, representing the two resistance strategies, will provide a better understanding of their behaviour and biochemical and physiological mechanisms driving drought resistance in field conditions in this semi-arid regions.

In the present work, we hypothesize that two native shrubs from Patagonian shrublands with contrasting resistance mechanisms to dry periods display different morphological, biochemical (photosynthetic pigment content) and physiological (endogenous level of phytohormones) traits in different seasons. We performed a field study with seasonal comparisons between a drought-tolerant species (*L. divaricata*) and a drought-avoiding species (*L. chilense*) to elucidate new morpho-histological, biochemical and physiological traits that contribute to drought resistance strategies.

2. Materials and methods

2.1. Study site

The study was performed in the wildlife refuge “La Esperanza” of the Natural Patagonia foundation, which is located in the northeast section of Chubut province and has an area of 67 km², coordinates: 42°7'43.92" S and 64°57'40.99" W Fig. 1a describe the location of the Monte phytogeographic province in Argentina according to Fernández and Busso (1999), Fig. 1b shows the specific location and Fig. 1c shows a picture of the study site. This region belongs to the Patagonian rangelands of Argentina, which are characterized by a semi-arid climate with annual rainfall below 250 mm, generally concentrated in winter and/or spring, and low water availability during summer and/or autumn (Cabrera, 1976).

2.2. Plant species selection

The selected species are native to the Patagonian rangelands. *Larrea divaricata* Cav (Fig. 1e). has been described as an evergreen shrub, with a developed leaf phenology that is not interrupted during the dry period, regardless of the soil water availability. *Lycium chilense* Miers ex Bert (Fig. 1f). has been described as a deciduous shrub that loses its leaves during dry seasons (frequently during autumn and winter) and produces new leaves in spring and flowers when the water availability increases (Bertiller et al., 1991; Campanella and Bertiller, 2008; Varela et al., 2016). Typical patches from the Patagonian Monte are composed of *L. divaricata* (a tall evergreen shrub, between 1.5 and 1.85 m in height) and the grasses around this species, and other patches are composed of *L. chilense* (a tall deciduous shrub, between 0.8 and 1.3 m in height), which is located in the centre of a patch dominated by *Chuquiraga avellanadae* (Fig. 1a–c).

Larrea divaricata and *Lycium chilense* provide various ecological services. These species constitute the refuge for native herbivorous (Cenzano et al., 2016), prevent soil degradation processes (Chartier and Rostagno, 2006), and are used as tinctorial and fuel wood (Ladio and Lozada, 2009). *L. chilense* is also used as fodder, and its fruits are edible, whereas *L. divaricata* is also used in medicine, veterinary practice, vinegar production and construction (Ladio and Lozada, 2009).

2.3. Rainfall and soil water content

During the study period (May 2011–February 2012), we registered the daily precipitation with an automatic data recorder (21 × Micrologger, Campbell Scientific) located at the study site. Material was sampled in autumn (May 25, 2011), winter (August 20, 2011), spring (November 26, 2011) and summer (February 18, 2012). Five soil cores (5.5 cm in diameter and 30 cm in depth) were taken (Fig. 1d) below five randomly selected patches, subdivided at three depths (10, 20 and 30 cm) and placed in plastic bags to prevent moisture loss. The soil water content (SWC) was measured through a gravimetric method. The wet weight (WW) and dry weight (DW; after oven drying at 105 °C for 48 h) were measured. The percentage of the soil water content was calculated using the following equation:

$$\text{Soil moisture content (\%)} = (\text{WW} - \text{DW}) / \text{DW} \times 100$$

2.4. Plant material

Samples were collected during each season: autumn, winter, spring and summer (2011). Five samples (five plants per species per season) with the same floristic composition and similar height were randomly selected from five patches. All plants were found at the mature development stage.

Green leaves from *L. chilense* were collected during spring and summer because this species is deciduous and loses its leaves during dry periods (autumn and winter in our study). Small branches with fully expanded green leaves and fine roots (< 2 mm in diameter) were collected in the field, immediately dipped in liquid nitrogen in the field and transferred in a cold and dark chamber connected electrically to a vehicle battery to the laboratory. The samples were maintained at –80 °C until lyophilisation. Fine roots were collected from topsoil to a depth of 60 cm. The dry weight (DW) of green leaves and fine roots was determined after 72 h of lyophilisation, and the DW of green leaves was obtained after collecting all fully expanded green leaves for each sample from one plant.

2.5. Relative water content (RWC)

Samples of fully expanded leaves and fine roots were placed into hermetic vials to prevent moisture loss, and the relative water content

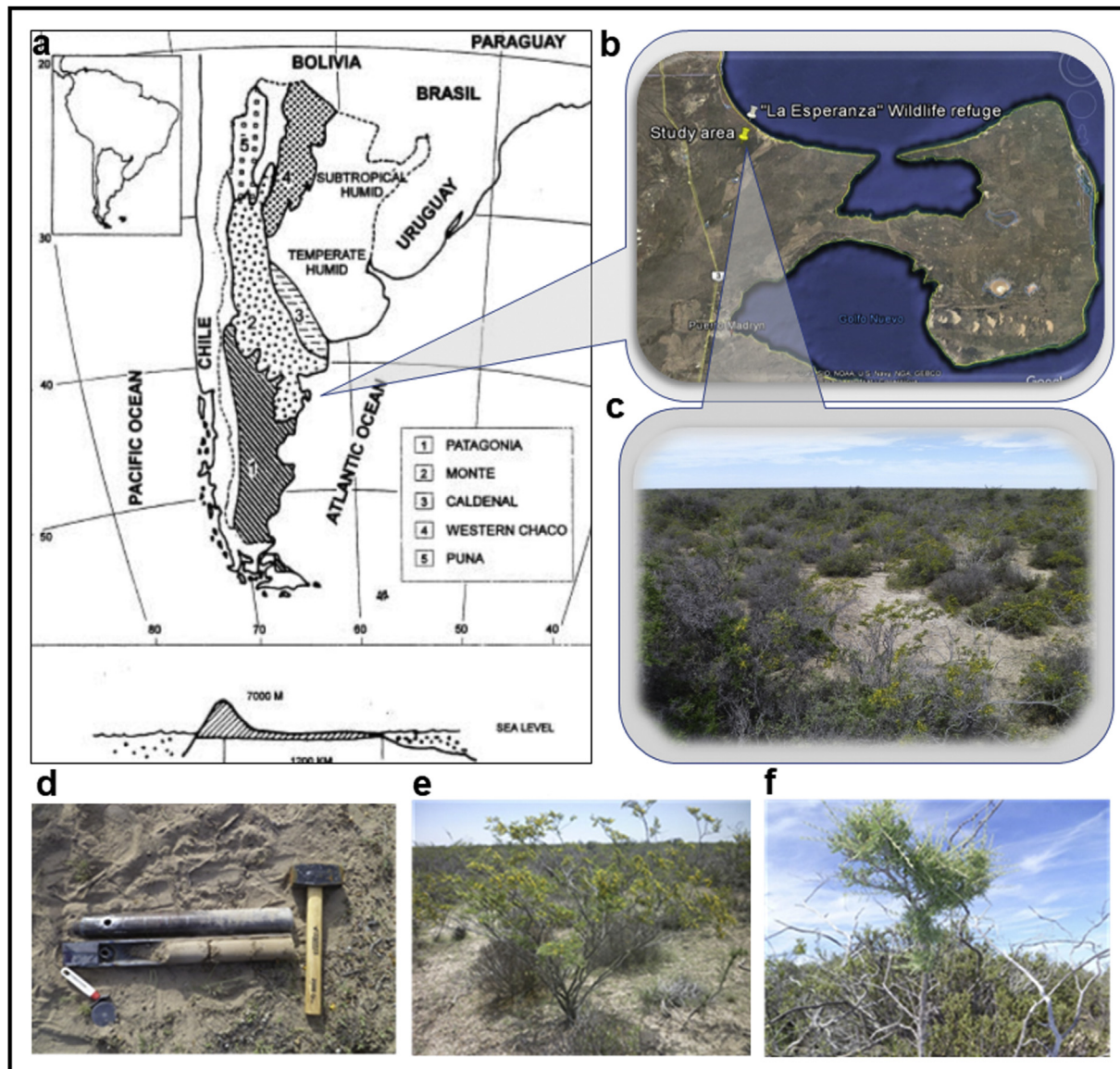


Fig. 1. a) Location of the Monte phytogeographic province in Argentina. b) Location of the study site, approximately 75 km north of Puerto Madryn city, Chubut Province. c) General view of the sampling site in the wildlife refuge “La Esperanza”. d) Soil sampling at three depths (10, 20 and 30 cm). Native species of the Patagonian rangelands: e) *Larrea divaricata* Cav. and f) *Lycium chilense* Miers ex Bert.

(RWC) was calculated (Xu and Zhou, 2007). The fresh weight (FW), weight at full turgor (TW; in water for 24 h), and dry weight (DW; after drying in an oven at 80 °C for 24 h) were measured. The RWC was calculated using the following equation:

$$\text{RWC} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100$$

2.6. Anatomical analysis

Five samples of fully expanded green leaves and fine roots from each plant at each season were selected.

The material was fixed in FAA solution (ethanol 50%, water 35%, formaldehyde 10% and acetic acid 5%) and stored in the same solution at room temperature until analysis. Free-hand sections were prepared, stained with safranin and mounted on a slide with 10% glycerol. Photomicrographs were taken using a Zeiss Axiophot microscope, and the images were captured and digitized using an AxioCam HRC camera and the AxioVision 4.3 programme. The characteristics recorded for leaves were the thickness and width of the leaf blade, the distance

between vascular bundles and the number of vascular bundles. The characteristics recorded for roots were the total root radius, xylem tissue cylinder, phloem width and cortex parenchyma width. Measurements were made from different transverse-section photographs using Image-Pro-plus 4.5 software.

2.7. Photosynthetic pigment content

The chlorophyll *a* (Chl *a*) and *b* (Chl *b*) and carotenoids (Car) contents in green leaves were measured. The pigments were determined spectrophotometrically following the method proposed by Vernon (1960) and McKinney (1941).

2.8. Plant hormone determination

2.8.1. Extraction and purification

Plant hormones were extracted and purified as described by Zhou et al. (2003), with some modifications. Leaf and root tissue (200 mg dry weight) were ground in a mortar with liquid nitrogen. ABA, ABA-GE,

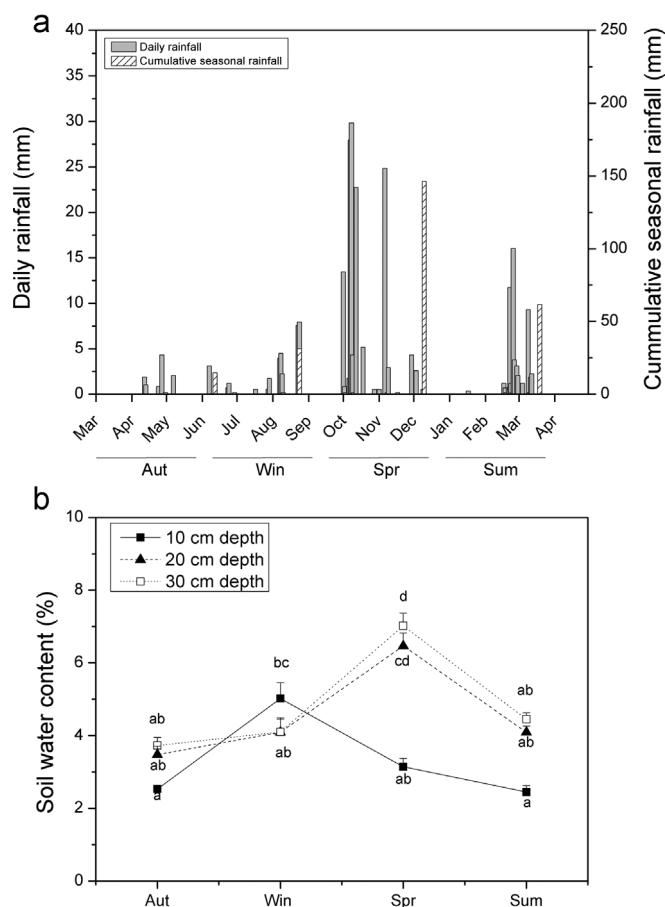


Fig. 2. a) Daily and cumulative rainfalls in each season: autumn (Aut), winter (Win), spring (Spr) and summer (Sum). b) Soil water content at three depths (10 cm, 20 cm, 30 cm) in each season: autumn (Aut), winter (Win), spring (Spr) and summer (Sum). The values are the means \pm s.e. ($n = 5$). Different letters indicate significant differences between the seasons and depths ($P < 0.05$).

GAs (GA_1 and GA_3) and auxin (IAA) were extracted with 5 ml of extraction buffer at pH 2.8, and 50 ng/sample of 2H6-ABA, 2H5-ABA-GE, 2H2- GA_1 , 2H2- GA_3 and 2H5-AIA (OlChemIm, Ltd., Olomouc, Czech Republic) were added as internal standard. The extracts were centrifuged at 8000 rpm for 15 min, and the supernatants were collected and mixed with ethyl acetate. The organic phase was extracted and evaporated at 37 °C. The dried extracts were dissolved in 100 μ l of methanol. This samples had the phytohormones extracted and the internal standard (0.5 ng/ μ L). A 10- μ l volume of each sample was injected onto a liquid chromatography (LC) instrument with electrospray ionization (ESI) (Waters Corp., New York, NY, USA).

2.8.2. Liquid chromatography

Analyses were performed using an Alliance2695 (LC Separation Module, Waters, USA) quaternary pump equipped with an auto-sampler. A Restek C18 (Restek, USA) column (2.19 \times 100 mm, 5 μ m) was used at 28 °C. For the elution gradient, a binary solvent consisting of 0.2% acetic acid in H₂O (solvent B) and MeOH (solvent A) was applied at a constant flow rate of 200 μ l min⁻¹. A linear gradient profile with the following proportions (v/v) of solvent A was applied: [t (min), % A]: (0, 40) and (25, 80), with 7 min for re-equilibration.

2.8.3. Mass spectrometry

MS/MS experiments were performed on a Micromass Quattro UltimaTM PT double quadrupole mass spectrometer (Micromass, Manchester City, UK). All analyses were performed using a turbo ion spray source in the negative ion mode with the following settings:

capillary voltage, -3000 V; energy cone, 35 V; RF Lens1 (20); RF Lens2 (0.3); source temperature, 100 °C; solvation temperature, 380 °C; gas cone, 100 l h⁻¹; collision (50), and multiplier (650).

The MS/MS parameters were optimized through infusion experiments using individual standard solutions of each hormone at a concentration of 50 ng/ μ l diluted in mobile phase A/B (40:60, v/v). The MS/MS product ions were produced by collision-activated dissociation of selected precursor ions in the collision cell of the double quadrupole mass spectrometer, and the mass was analysed using the second analyser of the instrument. In the negative mode, the spectrum for each hormone yielded deprotonated molecules [M-H]⁻. Quantification was performed through the injection of samples in the multiple reaction monitoring (MRM) modes because multiple compounds can present the same nominal molecular mass. A combination of the parent mass and unique fragment ions was used for the selective monitoring of hormones in plant extracts. MRM acquisition was performed by monitoring ABA and 2H6ABA at 263 > 153 and 269 > 159, ABA-GE and 2H5-ABA-GE at 425 > 263 and 430 > 268, GA_1 and 2H2- GA_1 at 348 > 242 and 350 > 244, GA_3 and 2H2- GA_3 at 345 > 221 and 347 > 223, and AIA and 2H5-AIA at 175 > 130 and 180 > 135, respectively, with a dwell time of 1000 ms for each transition. LC-ESI-MS/MS limit of detection (LOD) was 4 mg g⁻¹ and limit of quantification (LOQ) was 0.1 mg. The data were acquired and analysed using MassLynxTM 4.1 and QuanLynxTM 4.1 (Micromass, Manchester, UK) software. For quantification, values were obtained from a calibration curve previously constructed using each hormone (ABA, ABA-GE, GAs and AIA) and their pure standard/deuterated internal standards (Sigma, St. Louis, MO, USA).

2.9. Statistical analysis

Infostat (2011 v.), Statgraphics Centurion XVI and SPSS 15.0 software were used. One-way ANOVA was used to compare the soil water content between seasons. A Kruskal Wallis non-parametric test was used to compare the rainfall and morphohistological traits between seasons. One-way ANOVA was used to compare the results of the biochemical analyses of *L. divaricata* and *L. chilense* between seasons. Tukey's test was used for comparisons of the means, and the differences were considered significant if $P < 0.05$. The data were tested for normality by the Shapiro-Wilk test and for homogeneity of variance by Levene's test. If the ANOVA assumptions were not correct (homogeneity of variance and normal distribution of the residuals), a natural logarithm transformation was applied.

A multivariate analysis was performed using all data obtained from the biochemical analyses. A linear discriminant analysis (LDA) was performed to reduce dimensionality, i.e., maximizing the variance between the categories (season/species for all the data together and season/tissue within each species) and minimizing the variance within categories, with the aim of identifying the functional groups defined according to drought resistance mechanisms (tolerance or avoidance).

3. Results

3.1. Rainfall data and soil water content

During the sampling period, the daily rainfall did not exceed 30 mm, and the cumulative seasonal rainfall showed a significant increase in spring (146 mm) compared with the other seasons (Fig. 2a). However, in autumn, a cumulative rainfall of only 14.6 mm was registered, and intermediate values were registered in winter (31.2 mm) and summer (61.54 mm). The total rainfall during the study year was 253.75 mm. The highest soil water content (SWC) was in spring, specifically at a depth of 20 and 30 cm, which is consistent with the rainfall values (Fig. 2b). For a better understanding of the terms used in this study, spring, autumn, and winter/summer were classified as the "wet season," "dry season," and "intermediate rainfall seasons," according to

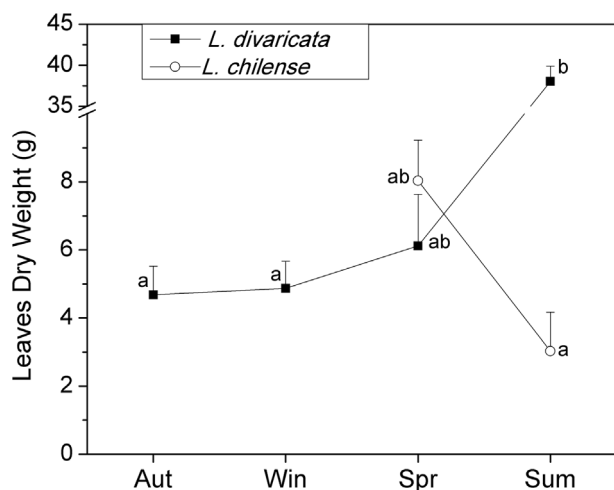


Fig. 3. Leaf dry weights of *Larrea divaricata* (perennial plant) during autumn (Aut), winter (Win), spring (Spr) and summer (Sum) and *Lycium chilense* (deciduous plant) during spring (Spr) and summer (Sum). The seasons were compared by two-way ANOVA. The values are the means \pm s.e. ($n = 5$). Different letters indicate significant differences between seasons and species ($P < 0.05$).

the rainfall and soil water content that was observed.

3.2. Green leaf dry weight and relative water content

The green leaf dry weights of both species were similar in all of the seasons with the exception of summer (the season after rainfall), when *L. divaricata* showed higher values (Fig. 3). The RWC values in the leaves of *L. divaricata* increased significantly during summer (52.9–72.8%, Fig. 4). The RWC values of the *L. chilense* leaves increased from spring to summer, and the metabolism in these leaves was dependent on water availability (Fig. 4).

The RWC values of the *L. divaricata* roots (48.4–65.5%) were similar, with a slight increase during the dry seasons (winter and autumn; Fig. 4). The *L. chilense* roots had RWC values in the range of 24.4–49.2% (Fig. 4).

3.3. Morphohistological traits

Images from transverse sections were used to measure the

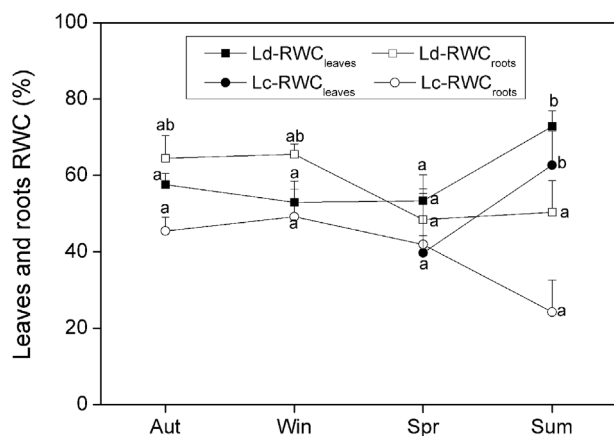


Fig. 4. Relative water content (RWC) in green leaves and fine roots of *Larrea divaricata* and *Lycium chilense* in the four seasons: autumn (Aut), winter (Win), spring (Spr) and summer (Sum). The values are the means \pm s.e. ($n = 5$). Different letters indicate significant differences between seasons and species ($P < 0.05$).

morphohistological traits (Fig. 5). Table 1 shows the results obtained for the analyses of the roots and leaves from free-hand sections.

The anatomical analysis of green leaves of *L. divaricata* showed that the size differed between summer and winter, but the variation in the amount of vascular bundles was not significant, despite the greater distance between them due to the size variation. The *L. divaricata* leaf blades increased in thickness from autumn to summer, whereas the width increased from spring to summer. The vascular bundle distance increased in spring and summer, but the number of vascular bundles did not differ between the seasons. *L. chilense* showed no differences in the various leaf traits measured (thickness, width, distance between vascular bundles and number of vascular bundles) between the two seasons analysed (spring and summer).

In *L. divaricata*, all measured root traits showed increased values in winter compared with the other seasons. The *L. chilense* roots had a larger radius in winter than in the other seasons, whereas the cortex parenchyma width and phloem width did not differ between the seasons.

3.4. Photosynthetic pigments content

Both species had higher chlorophyll *a* and *b* contents during spring, in parallel with the higher rainfall and the increased carotenoid content detected during spring and summer (Fig. 6).

3.5. Plant hormone content

The ABA endogenous content was significantly higher in green leaves of *L. divaricata* than in those of *L. chilense* during summer (Fig. 7a). In *L. divaricata* green leaves, the ABA content increased in spring and was highest in summer, whereas the *L. chilense* leaves showed the highest ABA content in spring. In contrast, The ABA-GE content was not significant different in *L. divaricata* during the four seasons even though the average value was double in winter than in summer. The bioactive GAs measured were GA₁ and GA₃. The *L. chilense* leaves had a higher GA₁ content than *L. divaricata*, and the content of this hormone was higher in spring than in summer in the former species. In the leaves of *L. divaricata*, the GA₃ content did not varies during the seasons, whereas in *L. chilense*, the GA₃ content was highest in summer (Fig. 7b). Finally, *L. divaricata* leaves had a higher IAA content in summer than those of *L. chilense* (Fig. 7c). In the fine roots of *L. divaricata*, the ABA and ABA-GE contents did not shows significant differences between the seasons and the species even though the average value decrease from winter to autumn and increase double from winter to summer. Also, the ABA content in *L. chilense* was higher in spring than in summer with no significant differences (Fig. 8a). The amount of GAs in roots was higher than that in the leaves, and *L. chilense* had higher GA₁ content than *L. divaricata* in summer (Fig. 8b). The IAA content was increased in autumn in both species and in summer in *L. chilense* roots (Fig. 7b). Finally, it is important to clarify that, because the individuals are randomly selected in the field, the variation of the value could be influenced by external factors that cannot be controlled. Therefore, the standard deviation of the statistical analysis is too high to show significant differences and we focus the discussion section in the fold change of the average values.

3.6. Linear discriminant analysis

A LDA was performed to visualize differences among combinations of season and species. Additionally, because the univariate analysis could be affected by external factors (explained above), the multivariate analysis helps to detect the influence of seasonal changes in the different drought resistance strategies. For green leaves, the LDA analysis showed that the values corresponding to *L. divaricata* (Ld) in summer were separated from the other values by CA 1, and CA 2 separated the seasons (Fig. 9a). It is important to remember that during

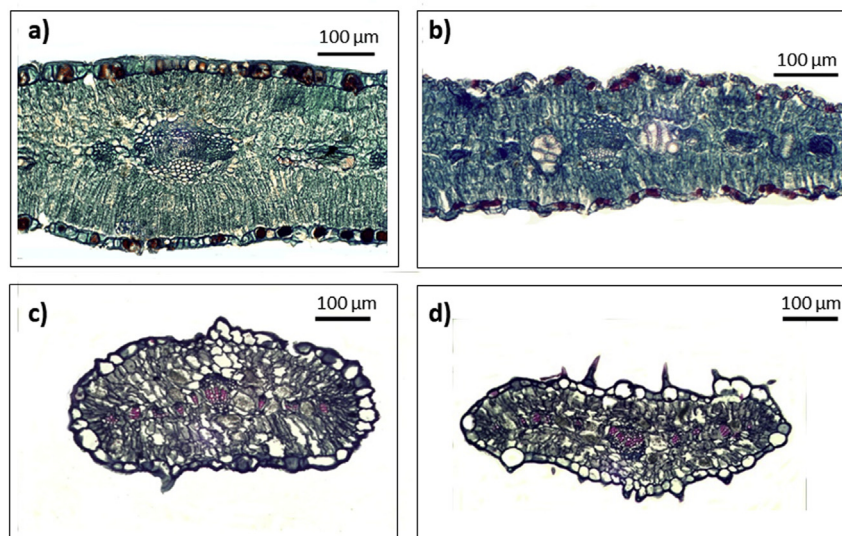


Fig. 5. Transverse sections photographs of a) *Larrea divaricata* in spring, b) *Larrea divaricata* in autumn c) *Lycium chilense* in spring and d) *Lycium chilense* in autumn.

Table 1

Leaf and root traits measured in photographs of free-hand sections in different seasons.

Species	Leaf traits	Autumn	Winter	Spring	Summer
<i>L. divaricata</i>	Thickness(μm)	3622.4 ± 19.5 a	3581.3 ± 22.05 b	4907.9 ± 80.7b	5179.6 ± 37 b
	Width (μm)	310.3 ± 4.3 ab	279.5 ± 28.01 a	374.07 ± 21.9 bc	398.4 ± 9.7 c
	Distance between vascular bundles (μm)	201.08 ± 17.71 a	206.3 ± 10.54 a	298.7 ± 13.7 b	321.03 ± 31.4 b
	Number of vascular bundles	20 a	20 a	21 a	20 a
	<i>L. chilense</i>	Thickness (μm)	–	–	1450.81 ± 36.19 a
	Width (μm)	–	–	585.01 ± 22.69 a	605.36 ± 16.51 a
	Distance between vascular bundles (μm)	–	–	118.91 ± 11.12 a	113.95 ± 13.42 a
	Number of vascular bundles	–	–	10 a	9 a

Species	Root traits	Autumn	Winter	Spring	Summer
<i>L. divaricata</i>	Total radius (μm)	142.23 ± 15.64 b	165.67 ± 10.52 b	132.05 ± 3.42 b	89.81 ± 0.64 a
	Xylem tissue cylinder (μm)	94.99 ± 0.36 b	106.07 ± 8.45 b	85.41 ± 4.2 b	56.16 ± 1.06 a
	Phloem width (μm)	5.62 ± 0.65 a	10.73 ± 1.33 b	8.17 ± 1.04 ab	5 ± 0.49 a
	Cortex parenchyma width (μm)	17.53 ± 3.27 ab	21.56 ± 0.97 b	21.35 ± 3.36 ab	12.91 ± 0.78 a
<i>L. chilense</i>	Total radius (μm)	377.73 ± 25.53 a	498.07 ± 18.35 b	365.24 ± 19.13 a	401.87 ± 21.65 ab
	Xylem tissue cylinder (μm)	204.42 ± 15.35 a	247.69 ± 9.66 b	196.15 ± 19.67 a	203.56 ± 17.45 a
	Phloem width (μm)	50.67 ± 4.47 ab	129.93 ± 45.90 a	32.17 ± 6.58 a	43.76 ± 7.53 ab
	Cortex parenchyma width (μm)	48.56 ± 3.2 a	50.98 ± 2.75 a	46.67 ± 1.98 a	41.67 ± 2.46 a

Different letters indicate significant differences between the seasons according to a Kruskal-Wallis test ($P < 0.05$).

autumn and winter, *L. chilense* loses its leaves as a mechanism to adapt to dry periods, whereas *L. divaricata* maintains its leaves, instead changing its behaviour and metabolism to tolerate dry periods. Thus, the data corresponding to *L. divaricata* during autumn and winter were not included, and a slight overlap in the species data in the different seasons was found when both species had their leaves (i.e., spring and summer). The multivariate analysis showed that in summer, the green leaves from *L. divaricata* showed higher values for leaf dry weight, blade leaf thickness and ABA content compared with those of *L. chilense* (Fig. 9a).

Although the root analysis revealed similarities between the species (except during autumn, Fig. 9b), the multivariate analysis showed that the fine roots from *L. divaricata* had higher RWC and IAA levels during the autumn-dry period but similar levels of these variables during the winter and spring compared with those of *L. chilense* (Fig. 9b).

4. Discussion

In this study, we evaluated the morpho-histological, biochemical and physiological traits of two native shrubs to associate their resistance mechanisms with water availability in the different seasons throughout the year. At first, we found that, during the sampling period, the low soil water content was correlated with the scarce rainfall, not exceeding 30 mm per day, in agreement with previous reports for semi-arid regions (Cabrera, 1976). Interestingly, the soil water content was higher in the deepest samples in all the seasons, except for winter. This variation could be attributed to the rainfall events prior to the sampling days not having yet infiltrated into a greater depth (Grayson et al., 1997).

Desert plants are characterized by a suite of morphophysiological traits related to drought tolerance or drought avoidance (Chapin et al., 1993). In our study, *L. divaricata* had leaves in all seasons, whereas *L. chilense* lost its leaves during autumn and winter, producing new leaves

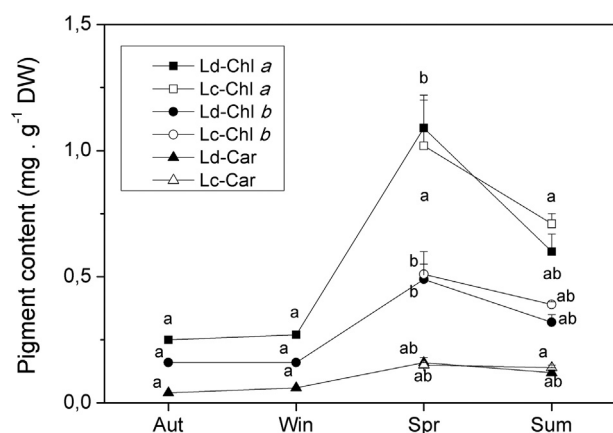


Fig. 6. Pigment content in green leaves of *Larrea divaricata* and *Lycium chilense* in the four seasons: autumn (Aut), winter (Win), spring (Spr) and summer (Sum). The values are the means \pm s.e. (n = 5). Different letters indicate significant differences between seasons and species ($P < 0.05$).

in spring and flowering during early summer. Also, the green leaf biomass of *L. divaricata* increased in summer, the season after the highest rainfall according to Yan et al. (2000) where biomass of *L. tridentata* increased in the season that follows the one with the highest rainfall recorded.

The RWC values in leaves and roots of *L. divaricata* suggest that this species is better adapted to the environment it inhabits based on its capacity to more efficiently regulate the water balance than *L. chilense*. This finding supports the notion that stressed plants have values lower than 30%, and the RWC of non-stressed plants ranges from 50 to 90% (Chapin et al., 1993). The smaller change in the RWC values in tolerant species might be due to osmotic adjustment mediated by ABA (Reddy et al., 2003; Tuteja and Sopory, 2008). However, the RWC increasing from spring to summer in *L. chilense* leaves, indicate a metabolism dependent on water availability correlated with the phenological stage (Bertiller et al., 1991), strategy previously described for drought-avoiding plants (Novoplansky and Goldberg, 2001; Bacelar et al., 2004).

Anatomical analyses of green leaves showed size variation in *L. divaricata*, whereas in *L. chilense*, no differences in the leaf size were observed in both seasons analysed. This size variation in evergreen species allows osmotic adjustment and increased tissue rigidity under low water availability conditions (Bacelar et al., 2006). We found that all root traits of *L. divaricata* and the root diameter of *L. chilense* showed higher values in winter compared with the other seasons, strategy described for water saving in deep soil improving water acquisition (Comas et al., 2013). Also, large root diameter has been associated with the production of lateral roots with transport and storage functions (Eissenstat et al., 2000; Baddeley and Watson, 2005). Therefore, we suggest that this small but significant increase in the root diameter in both shrubs and the higher values for phloem and cortex parenchyma widths in *L. divaricata* constitute strategies for coping with the winter-drought period and surviving.

In general, drought stress reduces photosynthesis and results in chlorophyll degradation (Zawaski and Busov, 2014), causing decreases in the pigment content (Reynolds et al., 2005; Liu et al., 2011). Consistent with these reports, we found low chlorophyll *a* and *b* and carotenoid contents in *L. divaricata* during dry periods. Also, as expected, both shrubs had higher levels of these pigments during spring, the more photosynthetically productive season.

Plants adjust their ABA levels continually in response to physiological and environmental changes. In plants grown under field conditions, the ABA level has been reported to increase in spring and summer, coinciding with the low relative water content in leaves and high temperature and solar radiation levels (López-Carbonell et al.,

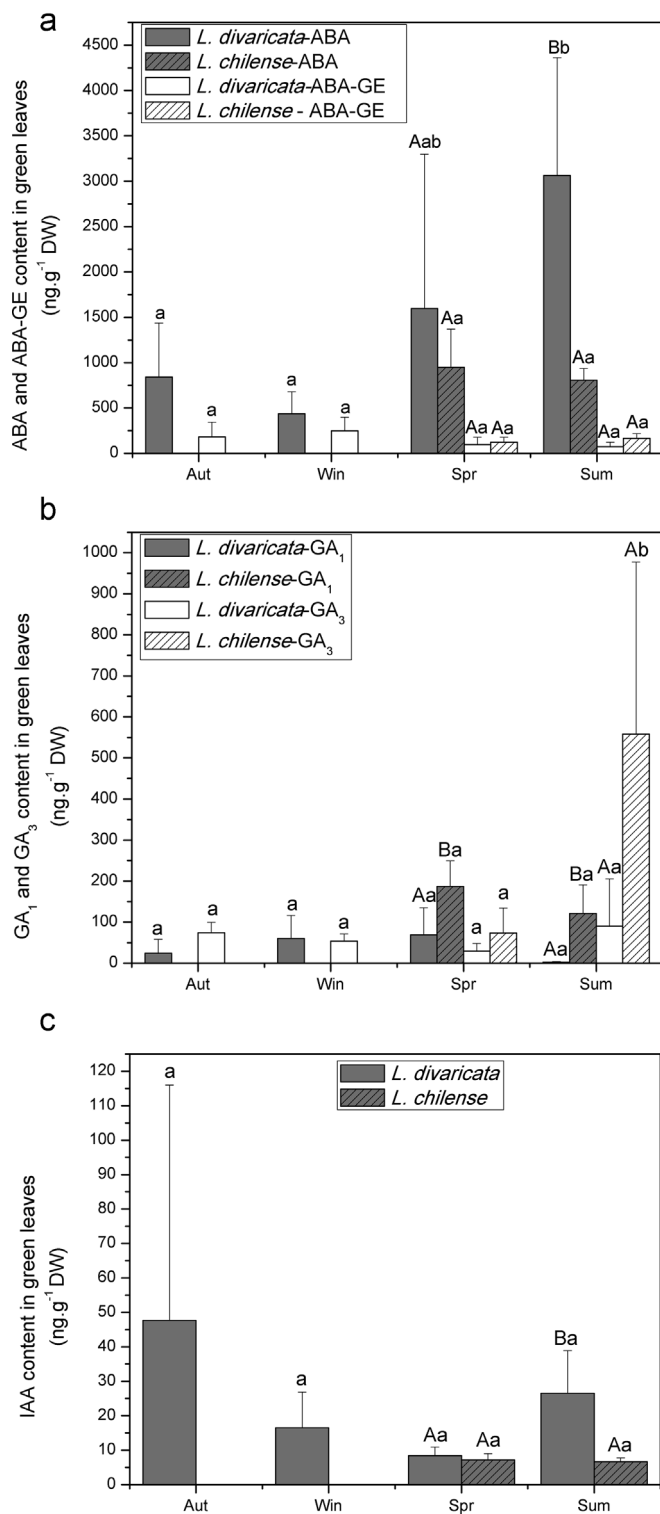


Fig. 7. a) ABA, ABA-GE, b) IAA, c) GA₁ and GA₃ content in green leaves of *Larrea divaricata* and *Lycium chilense* autumn (Aut), winter (Win), spring (Spr) and summer (Sum). The values are the means \pm s.e. (n = 5). Different uppercase letters indicate significant differences between species and different lowercase letters indicate significant differences between seasons for each hormone ($P < 0.05$).

2009). Consistent with this finding, our study, showed that the ABA content tended to increase in spring and summer, independently of the soil water availability. According to various researchers (Spollen et al., 2000; Sharp and Davies, 2009), endogenous ABA is necessary for the

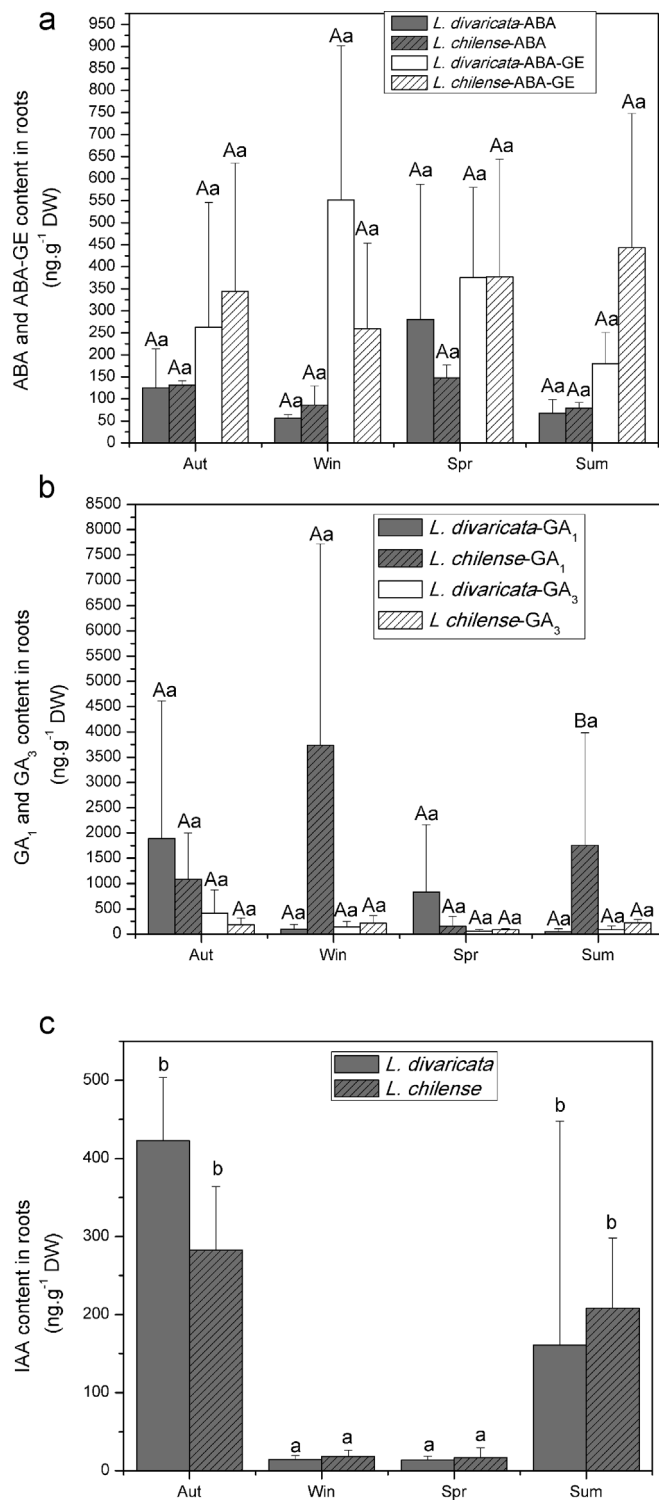


Fig. 8. a) ABA, ABA-GE, b) IAA, c) GA₁ and GA₃ content in roots of *Larrea divaricata* and *Lycium chilense* autumn (Aut), winter (Win), spring (Spr) and summer (Sum). The values are the means ± s.e. (n = 5). Different letters indicate significant differences between seasons for each specie and each hormone (P < 0.05).

maintenance of stem growth and leaf expansion, regardless of the water balance, suggesting a dual role for this hormone, acting as a growth promoter in non-stressful situations. The ABA results obtained support this notion suggesting that their main function is growth promotion. However, during summer, ABA induces stomatal closure (Sreenivasulu et al., 2012) allowing the maintenance of a high leaf RWC and

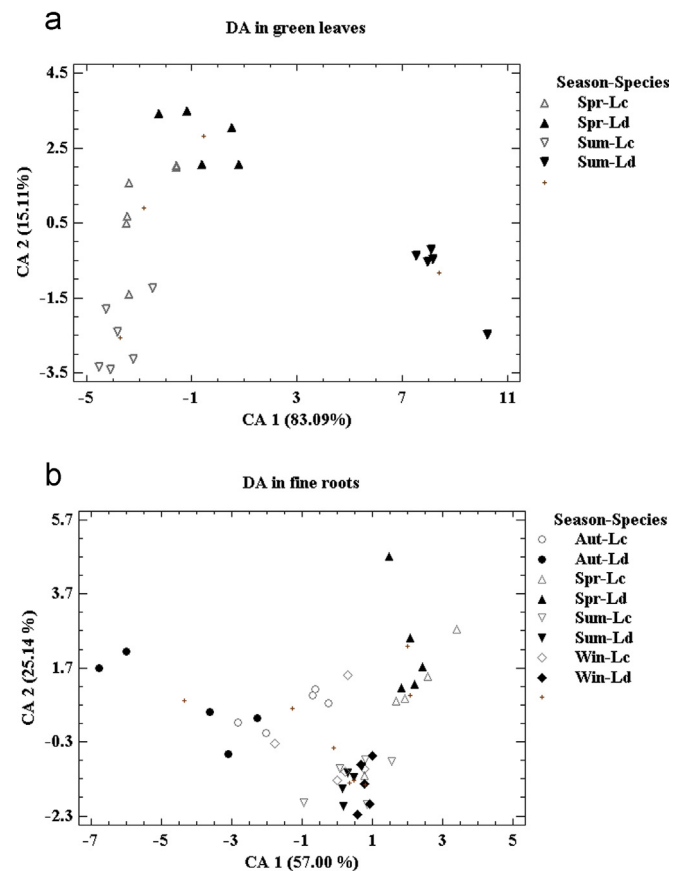


Fig. 9. Linear discriminant analysis of a) green leaves and b) fine roots. The variables refer to the combination of the species: *Larrea divaricata* (Ld-full and black dots) and *Lycium chilense* (Lc-empty and grey dots) with the four seasons: autumn (●/○), winter (◆/◇), spring (▲/△) and summer (▼/▽).

increasing the leaf dry weight. On the other side, the ABA-GE content did not change during the seasons, suggesting that ABA homeostasis is tightly controlled through its conjugation, with ABA-GE being both a transport and storage form of ABA (Sreenivasulu et al., 2012; Llanes et al., 2014). The involvement of GAs in drought responses has not been widely studied and has become an area of interest. Experiments with *A. thaliana* mutants have shown that plants with a reduced GA content are more tolerant to dry soil (Colebrook et al., 2014). In other words, high levels of active GAs would decrease the plant tolerance, thus, a growth promoter such as GA has to be maintained at low levels during water deficit. In our study, GAs content tends to increase in leaves during spring and summer showing that was not affected by the soil water availability and that their synthesis was only related to plant growth and development. In addition, the amount of GAs in roots was higher than in leaves suggesting that this hormone play a role in controlling shoot growth (Llanes et al., 2016).

In contrast, the increase in IAA in the roots of both species could be explained because this hormone accumulates in roots to maintain their growth, increasing root biomass and root branching to reach greater depths where water is available and hence conferring drought resistance (Dobra et al., 2010; Shi et al., 2014). In addition, the high IAA content found in roots during the dry period coincides with the low ABA level found in both species that might be correlated with the inability of ABA to suppress lateral root development (De Smet et al., 2006).

In our study, the hormonal profile suggested that (i) AIA and GAs play key roles in the regulation of seasonal growth independently of the water availability and (ii) ABA can play a dual role, specifically

maintaining normal physiology and controlling stomatal closure as well as prioritizing photosynthetic performance and rapid growth during the rainy season (Cenzano et al., 2016). Our results clearly demonstrate how coordinated interactions between phytohormones allow native plants to grow and survive under natural drought conditions.

Finally, LDA revealed significant differences in green leaf and roots traits between species across seasons. These differences were evident during summer (considered an intermediate rainfall season but showing the highest temperature and evaporation rates) and could be explained by the xerophytic traits of *Larrea divaricata* in contrast to the more mesophytic traits of *Lycium chilense* (Campanella and Bertiller, 2008; Varela et al., 2016; Bertiller et al., 1991).

5. Conclusions

Various morphological, biochemical and physiological aspects of two native shrubs from the Patagonian Monte, Argentina, which were analysed across different seasons, emerged as interesting findings from this study. During the dry season (autumn), the root traits of both species were separated into two groups depending on their drought resistance strategy. During the wet season (spring), green leaves and fine roots showed overlapping patterns. Finally, during summer, both species were separated by their green leaf traits (high ABA, ABA-GE, GA₁ and IAA contents) and by their fine root traits (GA₁ and GA₃ contents).

Our results support the notion that species with different drought resistance mechanisms (avoidance or tolerance) have different responses to dry periods throughout the year. *Larrea divaricata*, which displays more xerophytic traits, modifies its morphological traits (decreases its width leaf and the distance between leaf vascular bundles and increase the phloem and cortex parenchyma widths in roots) and maintains its physiological parameters (high RWC, high ABA levels in leaves during summer, high GA₃ in leaves during autumn, and high IAA in roots during autumn) to tolerate dry periods. *Lycium chilense*, which shows more mesophytic traits, has strategies to avoid dry periods (short vegetative growth duration before the dry period, loss of leaves during autumn and winter, high RWC in leaves, high ABA-GE and GA₃ levels in leaves during summer, high GA₁ and GA₃ contents in roots during summer, and high IAA content in roots during autumn and summer), showing a metabolism that is dependent on water availability for its growth. These traits provide important information regarding the resistance mechanisms used by each species to face dry periods. Therefore, the final developmental output is determined by a complex network in which the activity of one hormone is modulated by other hormonal pathways. Thus, metabolism and signalling pathways that control phytohormonal production, signal perception and the transduction of these molecules lead to the creation of a hormonal complex crosstalk that has just begun to be understood.

Contributors

This work was supported by the National Research Council of Argentina (CONICET) under a doctoral fellowship to C.V. and under grants from Projects D3148, PIP- P191 CONICET and SCTI of the Ministry of Science, Technology and Innovation of Chubut Province to A.C.

Notes on contributors

AMC, MVL and MCV originally formulated the idea. MCV and AMC conducted the fieldwork, and MCV performed the laboratory determinations. MCV and HR conducted the morpho-histological analyses. MCV and AMC analysed the data. MCV and AMC wrote the manuscript. MVL reviewed the Manuscript.

Acknowledgements

The authors thank Dr. Analia Carrera, Dr. Victoria Campanella, Soledad Martinez, Agostina Revellino and Marina Muñoz for their help in the field. Dr. Analia Carrera for providing the weather data used in this study and Dr. Agustina Tagliolegna for graphical design.

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